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UNDERSTANDING CELL PROLIFERATION AND MATERIAL-INDUCED CELL DEATH ON MICROFLUIDIC DEVICES MADE OF OFF-STOICHIOMETRIC THIOL-ENES

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ABSTRACT

This study addresses proliferation and long-term survival of cells on off-stoichiometric thiol-enes (OSTE), which have been recently associated with an inherent oxygen scavenging capability. The mechanistic basis of adverse impacts arising from free (excess) thiol monomers was examined to understand the framework for creation of material-induced hypoxia on-chip.

KEYWORDS: Polymer replication, off-stoichiometric thiol-enes, hypoxia, cell proliferation

INTRODUCTION

Since its introduction to fabrication of microfluidic devices [1], OSTE have gained increasing attention in rapid prototyping of a range of sample manipulation devices, including cell culture platforms [2]. Particularly the recent observation on inherent oxygen scavenging capability of thiol-rich OSTEs [2] may open up versatile new possibilities for organ-on-a-chip development by enabling creation of on-chip hypoxia *in situ*. Generally, OSTE curing can be done in the presence or absence of photoinitiators, e.g., by photolithography or casting against a mould, respectively [1,3]. Since many photoinitiators are cytotoxic, initiator-free casting appears as the most feasible approach to organ-on-a-chip microfabrication. However, initiator-free curing is prone to incomplete curing and thus leaching of excess monomers [4], which may interfere with the cell division-cycle.

EXPERIMENTAL

The cumulative population doubling level (cPDL) was determined for mouse embryonic fibroblasts (BALB-3T3) cultured on three differently UV-cured thiol-rich surfaces made from the commonly used tetrathiol and triallyl monomers, pentaerythritol tetrakis(3-mercaptopropionate) and 1,3,4-Triallyl-1,3,5-triazine-2,4,6 (1H,3H,5H)-trione, respectively. The monomers were mixed in a ratio yielding 50 mol-% excess of thiol functional groups and cured for 5 min either against a polydimethylsiloxane mold (native surface) or against the UV source (ambient surface). The impact of further heat-treatment (110°C, overnight) on the cell-compatibility of the native surface was also assessed. Total of 50 000 cells were seeded per each surface (Æ35 mm dish) and passaged every 7 days to calculate cPDL using Equation 1:

$$n = \frac{(log10\,F - log10\,I)}{0.031} \quad (1)$$

where *n* is the population doubling, *F* is the number of cells at the end of one passage and *I* is the number of cells that were seeded at the beginning of one passage. The proliferation rate was then quantified by bromodeoxyuridine (BrdU) assay using 4h incubation time (to allow BrdU incorporation into DNA) and flow cytometry analysis. To examine the possibility for the formation of reactive oxygen species in the presence of excess thiol monomers, trypsinized cells derived from conventional cell culture were incubated (10 min, 37°C) with Oxy-burst reagent (10 mM in phosphate-buffered saline) prior to their seeding onto the OSTE surfaces. Total of 400 000 treated cells were then incubated on differently cured OSTE surfaces and analyzed by flow cytometry using tert-butyl hydroperoxide (200 mM) as a positive control.

RESULTS AND DISCUSSION

Out of the three differently cured thiol-rich surfaces (Fig 1A), long-term (³ 56 days) cell survival was only achieved on ambient-exposed surfaces (Fig 1B). BrdU staining further strengthened these conclusions by showing that the cell proliferation rate on ambient-exposed surface was similar to the control for the first 4 weeks, and only slightly lower after 8 weeks (Fig 1C). Instead, on native surfaces, independent of the additional heat-treatment, the

cells failed to proliferate (Fig. 1B) suggesting that leaching monomers (arising from incomplete curing) may trigger material-induced cell death. Although heat-treatment has been reported to decrease the leaching of excess thiol monomers [2], it did not improve the long-term cell survival compared with native surfaces (Fig. 1B)

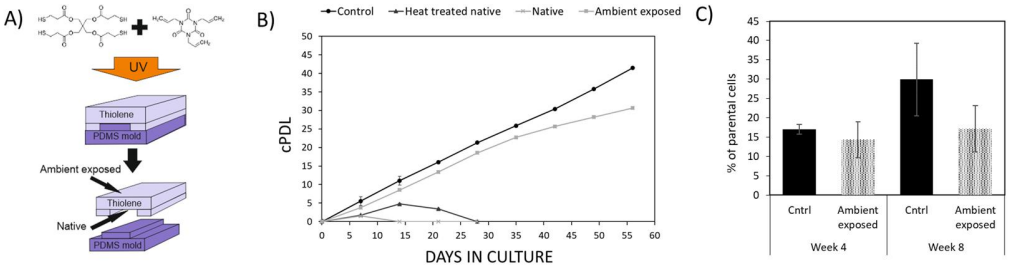

Figure 1: A) A schematic presentation of the UV-replica molding of OSTE made from tetrathiol and triallyl monomers illustrating the native and ambient-exposed surfaces. B) A cumulative population doubling level (cPDL) curve of BALB-3T3 cells on differently cured surfaces. C) The quantitative proliferation rate of BALB-3T3 cells grown on ambient-exposed surfaces after 4 and 8 weeks culturing in comparison to control (commercial Petri dish).

Further examination of the mechanistic basis of material-induced cell death on native and heat-treated surfaces gave no evidence on the formation of reactive oxygen species (Fig. 2A), which could have been possible side products of thiol oxidation. Nevertheless, a microfluidic chip design which combines hypoxia-inducing cover layer (native surface) with cell-compatible bottom layer (ambient-exposed) showed similar flawed cell morphology as what was observed in the presence of leaching monomers on native thiol-rich OSTE (Fig 2B-C). These results suggest that the material-induced cell death on native thiol-rich OSTE is triggered by direct monomer-induced damage to the cellular macromolecules, which typically results in early cellular senescence.

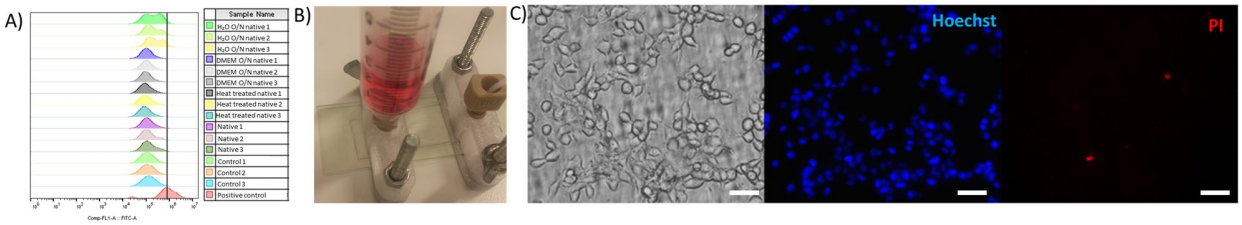

Figure 2: A) Quantitative analysis of BALB-3T3 cells labelled with Oxy-burst. Black line marks the intensity of the ROS-positive control, indicating no ROS formation in any of cell suspensions incubated with differently treated OSTE. B-C) Photograph of an OSTE chip with illustrations of flawed cell morphology (bright field), but no dead cells (PI stained) at the bottom of the microchannel due to thiol monomer leakage (24 hour incubation). Scale bars 50 µm.

CONCLUSION

With a view to controlling on-chip oxygen levels *via* thiol-induced hypoxia, oxidation of the leaching thiols has been hypothesized to play an important role. However, when designing OSTE-based organ-on-a-chips, the material-induced cell death associated with thiol monomers should be taken into account to ascertain that early cellular senescence arising from free thiol monomers is not to be mixed with that induced by hypoxia.

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